SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

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aaacttaccc cggaacaggc cgagcagatc aaaacgctac tgcaatacag cccatccagc 120

accaactccc agccgtggca ttttattgtt gccagcacgg aagaaggtaa agcgcgtgtt 180

gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgcac 240

gtcgtggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttgttgac 300

caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt 360

cgcaagttct tcgctgatat gcaccgtaaa gatctgcatg atgatgcaga gtggatggca 420

aaacaggttt atctcaacgt cggtaacttc ctgctcggcg tggcggctct gggtctggac 480

gcggtaccca tcgaaggttt tgacgccgcc atcctcgatg cagaatttgg tctgaaagag 540

aaaggctaca ccagtctggt ggttgttccg gtaggtcatc acagcgttga agattttaac 600

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<210> 2

<211> 826

<212> DNA

<213> Pseudomanas putida JLR11

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agcgatcttc ctgtggatga gcagatgctg agctgggcga tcgcggcggc ccagtcagcc 180

tcgacttcct cgaacctgca agcttggagc gtgctcgccg tgcgggatcg cgagcgtctc 240

gcgaggcttg cccgactgtc cggtaaccag cgccatgtcg agcaggcacc gctgttcctg 300

gtctggctcg tggactggtc acgcctacgc cgactagcca gaacccttca ggcaccgact 360

gcaggtatcg actatttaga aagctacacc gtcggtgttg tagatgcagc tctggccgct 420

cagaacgccg cactagcttt cgaggcccaa ggactgggaa tcgtttacat cggcggaatg 480

cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac tttcgctgta 540

tttggcatgt gcgtcggtca tcccgatccg gcacagcccg ccgagatcaa gccacgcctg 600

gcgcaatcag tggtgcttca ccgtgagcgc tatgaggcca ccgaggcaga ggcggtttca 660

gttgctgcct atgaccgaag gatgagcgac ttccaacatc gtcaacaacg cgaaaaccgt 720

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<210> 3

<211> 1066

<212> DNA

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

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<222> (996)..(1010)

<223> primer

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat    114
                               Met Gly Ser Ser His His His His His
                               1               5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act      162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10                  15                  20                  25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat      210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp
                30                  35                  40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc      258
Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala
            45                  50                  55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg      306
Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu
        60                  65                  70
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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt      354
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val
    75                  80                  85

gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt      402
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly
90                  95                  100                 105

aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg      450
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val
                110                 115                 120

gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt      498
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val
            125                 130                 135

gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa      546
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys
        140                 145                 150

gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa      594
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys
    155                 160                 165

gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac      642
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn
170                 175                 180                 185

gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta      690
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val
                190                 195                 200

ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg      738
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu
            205                 210                 215

aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac      786
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His
        220                 225                 230

agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa      834
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln
    235                 240                 245

aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggctat     888
Asn Ile Thr Leu Thr Glu Val
250                 255

ttcctctcag attctcctga tttgcataac cctgtttcag caagcttcgt catcataggc    948

tgctgttgaa gcttgcggcc gcactcgagc accaccacca ccaccactga gatccggctg   1008

ctaacaaagc ccgaaaggaa gctgagttgg ctgctgccac cgctgagcaa taactagc     1066
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<210> 4

<211> 256

<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> misc_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

<220>

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<222> (996)..(1010)

<223> primer

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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
            20                  25                  30

Gly Ser Cys Cys Cys Cys Cys Cys Asp Ile Ile Ser Val Ala Leu Lys
        35                  40                  45

Arg His Ser Thr Lys Ala Phe Asp Ala Ser Lys Lys Leu Thr Pro Glu
    50                  55                  60

Gln Ala Glu Gln Ile Lys Thr Leu Leu Gln Tyr Ser Pro Ser Ser Thr
65                  70                  75                  80
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```
Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys
                85                  90                  95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg
            100                 105                 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala
        115                 120                 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp
    130                 135                 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg
145                 150                 155                 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
                165                 170                 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly
            180                 185                 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala
        195                 200                 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser
    210                 215                 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala
225                 230                 235                 240

Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val
                245                 250                 255
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<210> 5

<211> 1221

<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

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<222> (88)..(1029)

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<222> (190)..(225)

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<222> (190)..(207)

<223> cys tag

<220>

<221> misc_feature

<222> (936)..(956)

<223> primer

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat    114
                               Met Gly Ser Ser His His His His His
                               1               5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act      162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10                  15                  20                  25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc      210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser
                30                  35                  40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca      258
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro
            45                  50                  55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat      306
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His
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        60                  65                  70

cga tca gta cgt gcc tac agc gat ctt cct gtg gat gag cag atg ctg      354
Arg Ser Val Arg Ala Tyr Ser Asp Leu Pro Val Asp Glu Gln Met Leu
    75                  80                  85

agc tgg gcg atc gcg gcg gcc cag tca gcc tcg act tcc tcg aac ctg      402
Ser Trp Ala Ile Ala Ala Ala Gln Ser Ala Ser Thr Ser Ser Asn Leu
90                  95                  100                 105

caa gct tgg agc gtg ctc gcc gtg cgg gat cgc gag cgt ctc gcg agg      450
Gln Ala Trp Ser Val Leu Ala Val Arg Asp Arg Glu Arg Leu Ala Arg
                110                 115                 120

ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg      498
Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu
            125                 130                 135

ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga      546
Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg
        140                 145                 150

acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc      594
Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr
    155                 160                 165

gtc ggt gtt gta gat gca gct ctg gcc gct cag aac gcc gca cta gct      642
Val Gly Val Val Asp Ala Ala Leu Ala Ala Gln Asn Ala Ala Leu Ala
170                 175                 180                 185

ttc gag gcc caa gga ctg gga atc gtt tac atc ggc gga atg cgc aac      690
Phe Glu Ala Gln Gly Leu Gly Ile Val Tyr Ile Gly Gly Met Arg Asn
                190                 195                 200

cac ccg gaa gcg atg tcc gag gag ctt ggc ctg cca aac gac act ttc      738
His Pro Glu Ala Met Ser Glu Glu Leu Gly Leu Pro Asn Asp Thr Phe
            205                 210                 215

gct gta ttt ggc atg tgc gtc ggt cat ccc gat ccg gca cag ccc gcc      786
Ala Val Phe Gly Met Cys Val Gly His Pro Asp Pro Ala Gln Pro Ala
        220                 225                 230

gag atc aag cca cgc ctg gcg caa tca gtg gtg ctt cac cgt gag cgc      834
Glu Ile Lys Pro Arg Leu Ala Gln Ser Val Val Leu His Arg Glu Arg
    235                 240                 245

tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga      882
Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg
250                 255                 260                 265

agg atg agc gac ttc caa cat cgt caa caa cgc gaa aac cgt tcc tgg      930
Arg Met Ser Asp Phe Gln His Arg Gln Gln Arg Glu Asn Arg Ser Trp
                270                 275                 280

tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga      978
Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly
            285                 290                 295

aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc     1026
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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg
300 305 310

tga gatagtgaga tatcccatgc ctattcccgc cgccctgaac cggagcacta 1079

atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc 1139

accaccacca ctgagatccg gctgctaaca aagcccgaaa ggaagctgag ttggctgctg 1199

ccaccgctga gcaataacta gc 1221

<210> 6

<211> 313

<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<222> (190)..(225)

<223> primer

<220>

<221> misc_feature

<222> (190)..(207)

<223> cys tag

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<222> (936)..(956)

<223> primer

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Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
20 25 30

Gly Ser Cys Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys
35 40 45

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Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
    50                  55                  60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
65                  70                  75                  80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala
                85                  90                  95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
            100                 105                 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
        115                 120                 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
    130                 135                 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
145                 150                 155                 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
                165                 170                 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
            180                 185                 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu
        195                 200                 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val
    210                 215                 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala
225                 230                 235                 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu
                245                 250                 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His
            260                 265                 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg
        275                 280                 285
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Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala
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Leu Asn Thr Leu Gly Phe Gly Leu Arg
305 310

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<211> 24

<212> DNA

<213> Escherichia coli

<400> 7
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<210> 8

<211> 27

<212> DNA

<213> Escherichia coli

<400> 8
cgtcatcata ggctgctgtt gaagctt 27

<210> 9

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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